

Cytological Studies in the Genus *Potamogeton* of Japan

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日本産ヒルムシロ属植物の細胞学的研究

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Introduction

From the view point of phylogeny, the genus *Potamogeton* has been studied by Raunkier (1896), Bennett (1896, 1904, 1905, 1907), Camus (1909), Chryster (1907), Fernald (1932), Hagström (1916), and Miki (1934, 1937).

According to Engler and Gilg (1924) the genus *Potamogeton* comprises 120 species in the world. In Japan 28 species have been found.

The cytological investigation of this genus has been made by only a few authors. Wiegand (1899) was the first to give some accounts of the chromosome number in some species. This investigation was followed by those of Wisniewska (1931), Kuleszanka (1934), Palmgren (1939), Takusagawa (1939), Harada (1942), Takusagawa (1950), and Harada (1956) the results obtained by these authors has shown that a Polyploid Condition exists in this genus. But most of the investigations were worked on the chromosome number of root-tip cells, and took no account of the behavior of the chromosome in the reduction division, karyotype, classification based upon karyotype.

In the present paper the results obtained from the observation of the behavior of chromosome in the reduction division in *P. M. Cs.* in 26 species and hybrid of the *potamogeton* as well as in some species which are taxonomically regarded as hybrids, are more fully given than in the previous papers. Moreover the discussion about the chromosome number, which not only as to the phylogenetical relationship among these species or hybrids, but also with regard to its karyotype, viewed from both the morphological and the biological standpoints is attempted.

It is my pleasant duty to record here a debt of gratitude to prof. N. Shimotomai of the Botanical Institute, Hiroshima University for his valuable suggestions and criticism throughout the work. My thanks are also due to prof. S. Miki of the Osaka City University who kindly placed the materials at my disposal.

Material and Methods

The following species and interspecific hybrid are dealt with in this work.

Potamogeton oxyphyllus, *P. panormitanus*, *P. pusillus*, *P. orientalis*, *P. monoginus*, *P. Fauriei*, *P. cristatus*, *P. vaseyi*, *P. kamogawaensis*, *P. numasakianus*, *P. apertus*, *P. Friyeri*, *P. malainoides*, *P. malaianus*, *P. dentatus*, *P. nipponicus*, *P. perfoliatus*, *P. crispus*, *P. praelongus*, *P. maackianus*, *P. biwaensis*, *P. distinctus*, *P. natans*, *P. anguillanus*, *P. alpinus* and *P. pectinatus*.

The materials used for microscopical investigations were taken from the plants, collected in

Koch prefecture, and from Shiga Prefecture and other several prefectures in Japan, and also from the plants cultivated for several years by Dr. S. Miki, in the experimental garden of the Boanical Institute of Kyoto University

Observations were made with root-tip cells for the somatic number of chromosomes and with pollen mother cells for the reduction division. Root-tips were fixed in Flemming's strong solution or its Bonn Modification and Nawaschins fixative (1% chromic acid 10 parts; glacialacetic acid 1 part; 40% formalin 4 parts¹). And Newton's gentian violet method was used for staining. Also root-tips were fixed in 0.002 mol. solution of 8-oxyquinoline, and the aceto-orcein smear method was used for staining. The pollen mother cells were fixed with Bouin's Fluid, Allen-Bouin, and Fleming's strong solution or its Bonn Modification. For pollen mother cells, the best results were obtained by using Bouin's solution (40% formalin 25 parts; saturated liquid of picric acid 75 parts; glacial acetic acid 5 parts) after a few minutes treatment in Carnoy's fluid (absolute alcohol 6 parts; chloroform 3 parts; glacial acetic acid 1 part). By the paraffin method all permanent preparations were made from the fixed material. Sections were cut 12–15 μ thick, and stained with Heidenhain's iron-alum haematoxylin. Newton's gentian violet method was used. The aceto-carmine smear method and permanent smear method were also used.

All figures of chromosomes were drawn with the aid of Abbe's camera lucida, and Zeiss 1/12 objective and Huyghens ocular 15X and 20X were used. These figures are magnified about 2,000 diameters.

Observation

I Orthoploids

A. Tetraploids

I. *Potamogeton cristatus* Regel. et Macck. $2n=28$

The somatic number of chromosomes is 28 in root-tip cells (Fig. 1a). These somatic chromosomes are slender. Of the 28 chromosomes 14 chromosomes are longer than the remaining 14, and 10 chromosomes of the 14 long chromosomes are slightly longer than the remaining 4, and 8 chromosomes of the 14 short chromosomes are shorter than the remaining 6. The karyotype of this species is as follows:

$$K(2n) = 28 = 10L_1 + 4L_2 + 6S_1 + 8S_2 \quad (\text{Fig. 1c})$$

(L chromosome is longer than S chromosome: L_1 longer than L_2 ; S_2 shorter than S_1)

At the first maturation division of this species, 14 bivalents are observed. Among them are 7 bivalents larger than the remaining 7, and 5 bivalents of the 7 large bivalent chromosomes slightly larger than the remaining 2, and 4 bivalents of the 7 small bivalent chromosomes slightly smaller than the remaining 3 (Fig. 1b).

The association of two components is nearly close. In the side view they show a dumb-bell-shape. At an earlier stage of the metaphase of first division all the bivalents make a normal equatorial plate.

In the next stage bivalents have completed their division and move apart on their way to the Pole. At the anaphase all the divided halves of bivalents move to different Poles. The first division is, therefore, reduction division. At the metaphase of the second division many figures show very regular plates.

In the succeeding stage a great number of chromosomes, forming the second division plate, divide longitudinally and pass to opposite poles.

1. Formalin was added immediately before use.

Most of the young microspores are normal in shape and size.

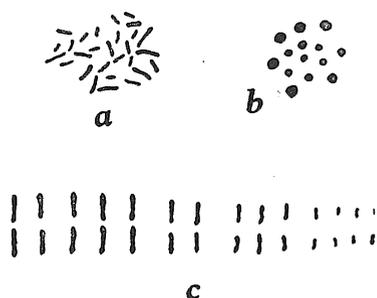


Fig. 1. *Potamogeton cristatus* Regel. et Maack

- a. Somatic nuclear plate showing 28 chromosomes.
- b. Heterotype metaphase in polar view showing 14 bivalent chromosomes.
- c. Somatic chromosomes. (ca. $\times 2000$)

All bivalent divide almost simultaneously in the anaphase and the divided halves move to opposite poles in a regular manner. Two daughter nuclei enter the interkinesis condition.

At the metaphase II all the chromosomes are regularly arranged on the equatorial plane in the metaphase, and at the anaphase each of the longitudinal halves of chromosome passes to the spindle poles.

The members of the pollen grains are uniform in shape and size.

P. limosellifolius Max., and *P. asiaticus* Benn. are the same species with *P. vaseyi* Robbins.

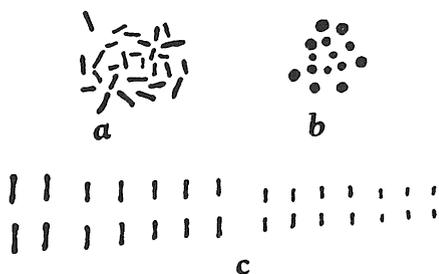


Fig. 2. *Potamogeton vaseyi* Robbins.

- a. Somatic nuclear plate showing 28 chromosomes.
- b. Heterotype metaphase in polar view showing 14 bivalent chromosomes.
- c. Somatic chromosomes. (ca. $\times 2000$)

of the 7 small bivalents chromosomes are slightly smaller than the remaining 4 bivalents chromosomes. (Fig. 3b)

All the chromosomes are round in shape. In the side view they show a dumb-bell-shape.

2 *Potamogeton vaseyi* Robbins. $2n=28$

The somatic number of chromosomes is 28 in root-tip cells, same number has been reported by Harada (1942). 14 chromosomes of the 28 are longer than the others and 4 chromosomes of the 14 long chromosomes are slightly longer than the remaining 10, and 6 chromosomes of the 14 short chromosomes are shorter than the remaining 8 (Fig. 2a)

Then the karyotype of this species is as follows:

$$K(2n)=28=4L_1+10L_2+8S_1+6S_2 \text{ (Fig. 2C)}$$

In P. M. Cs. 14 bivalent chromosomes are counted at diakinesis and metaphase I. Among them 7 bivalent chromosomes of the 14 are larger than the remaining 7, and 2 bivalent chromosomes of the 7 large bivalent chromosomes slightly larger than the remaining 5, and 3 bivalent chromosome of the 7 small chromosomes slightly smaller than the remaining 4 (Fig. 2b).

3. *Potamogeton monoginus* Miki. $2n=28$

The somatic number of the chromosome is 28, the same chromosome number as in the former species (Fig. 3a). The same number has been reported by Harada (1942). Among the 28 chromosomes 14 are longer than the others, of which 6 chromosomes are shorter than the remaining 8. The karyotype of this species is, therefore, denoted as follows:

$$K(2n)=28=14L_1+8S_1+6S_2 \text{ (Fig.3C)}$$

In P. M. Cs., 14 bivalent chromosomes are counted at he diakinesis and metaphase I and 14 univalent alents at the metaphase II. Among them 7 bivalents are larger than the remaining 7, and 3 bivalents

The division figures are, practically speaking, all regular both in the heterotype and the homotype division.

The pollen grains are mostly normal in appearance.

P. sibiricus Benn. is the same species as *P. monoginus* Miki. *P. zosteriforium* Sehum, and *P. porsildiorum* Fernald are nearly the same species as *P. monoginus* Miki.

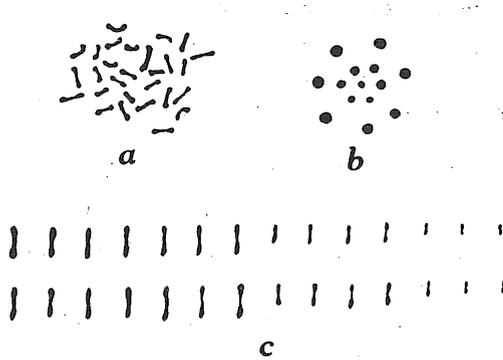


Fig. 3. *Potamogeton monoginus* Miki.
 a. Somatic nuclear plate showing 28 chromosomes.
 b. Heterotype metaphase in polar view showing 14 bivalent chromosomes.
 c. Somatic chromosomes. (ca. $\times 2000$)

4. *Potamogeton numasakianus* $2n=28$

This species has taxonomically been considered by Miki to be a hybrid between *P. cristatus* and *P. vaseyi*. This hybrid is intermediate in size and characteristics between those of *P. cristatus* and *P. vaseyi*. In this hybrid 14 chromosomes from *P. cristatus* and 14 chromosomes from *P. vascyi* are contributed so that 28 chromosomes would be expected as a somatic number. This was actually confirmed in root tip cells (Fig. 4 a). These somatic chromosomes are slender, 14 chromosomes of the 28 slightly longer than the remaining 14, and 4 chromosomes of 14 long chromosomes are slightly longer than the remaining 10, and 6 chromosomes of 14 short chromosomes are

shorter than the remaining 8. The karyotype of this species is as follows :

$$K(2n) = 4L_1 + 10L_2 + 8S_1 + 6S_2 \quad (\text{Fig. 4 d})$$

At the first maturation division of this species 14 bivalents are observed most frequently (Fig. 4b).

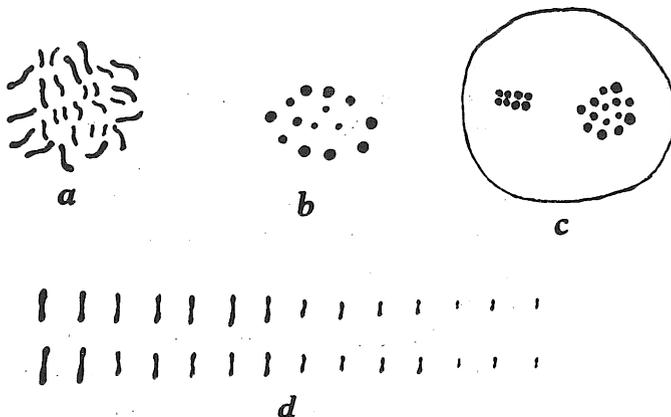


Fig. 4. *Potamogeton numasakianus* Benn.
 a. Somatic nuclear plate showing 28 chromosomes.
 b. Heterotype metaphase in polar view, showing 14 bivalent chromosomes.
 c. Metaphase of the second division.
 d. Somatic chromosomes. (ca. $\times 2000$)

Among them 7 bivalents are smaller than the remaining 7, and 2 bivalents of the 7 large bivalents are larger than the remaining 5, and 3 bivalents of the 7 small bivalents are smaller than the remaining 4.

The association of two components of bivalents is nearly as intimate as in the parents. Then the genomes of both parents seems to be nearly the same. In the side view they show dumb-bell shape.

At earlier stage of the metaphase I all bivalents make

a normal equatorial plate. In the next stage bivalents have completed their division and are moving apart on their way to the poles.

At the anaphase all divided halves of bivalents move to different Poles.

At the metaphase II many figures show very regular plates, two metaphase plates of daughter cells shown in Fig. 4c. In the succeeding stage a great number of chromosomes forming the second division plate, divide longitudinally and pass to opposite poles.

Most of the young microspores are normal in shape and size, but occasionally they show abnormal appearance. Giant and small microspores sometimes result.

B. Hexaploids

1. *Potamogeton apertus* Miki. $2n=42$

In this species 42 chromosomes are found in the root-tip cells, (Fig. 5a) and the same number has been reported by Harada (1942).

Among them 28 chromosomes are longer than the other, and 14 chromosomes of the 28 long chromosomes are slightly longer than the remaining 14.

The karyotype of this species is as follows :

$$K(2n)=42=14L_1+14S_1+14S_2 \quad (\text{Fig. 5c})$$

The chromosome behavior in the P. M. Cs. is on the whole regular in this species. The number of chromosome pairs is found in most cases to be 21 at the diakinesis and the metaphase in the heterotype as well as the homotype divisions (Fig. 5b); 7 bivalents of the 21 are slightly larger than the remaining 14, and 7 bivalents of the 14 small bivalents are slightly smaller than the remaining 7.

But this difference in size is not so distinct. In some cases of 1 or 2 bivalents the component chromosomes are very loosely associated.

In the second division the metaphase plates are very regular. The two plates are disposed at right angles or parallel to each other.

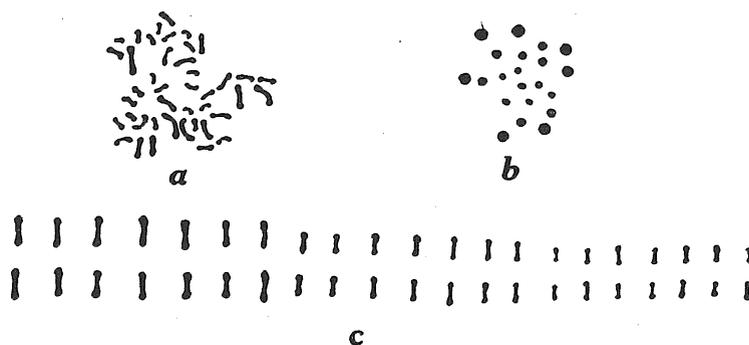


Fig. 5. *Potamogeton apertus* Miki.

a. Somatic nuclear plate showing 42 chromosomes.

b. Heterotype metaphase in polar view showing 21 bivalent chromosomes.

c. Somatic chromosomes. (ca. $\times 2000$)

2. *Potamogeton Friyeri* Benn. $2n=42$

The somatic chromosome number is 42 in this species (Fig. 6 a), 28 chromosomes of the 42 are longer than the remaining 14, and 14 chromosomes of the 28 long chromosomes are slightly longer than the remaining 14.

Then the karyotype of this species is as follows :

$$K(2n)=42=14L_1+14S_1+14S_2 \quad (\text{Fig. 6c})$$

At the metaphase I there are found 21 bivalent chromosomes; 7 bivalents of the 21 are slightly larger than the remaining 14, and 7 bivalents of the 14 small bivalents are slightly smaller than the remaining 7, but this difference in size is not so distinct (Fig. 6 b).

All the bivalents divide almost simultaneously at the anaphase, and the divided halves move to opposite poles in a regular manner. Two daughter nuclei enter the interkinesis condition.

In the second division all the chromosomes are regularly arranged on the equatorial plane in the metaphase, and in the anaphase each of the longitudinal halves of chromosomes passes to the spindle poles.

The number of the pollen tetrad, and pollen grains are uniform in shape and size. The maturation divisions are quite regular, and no significant irregularities have been found.

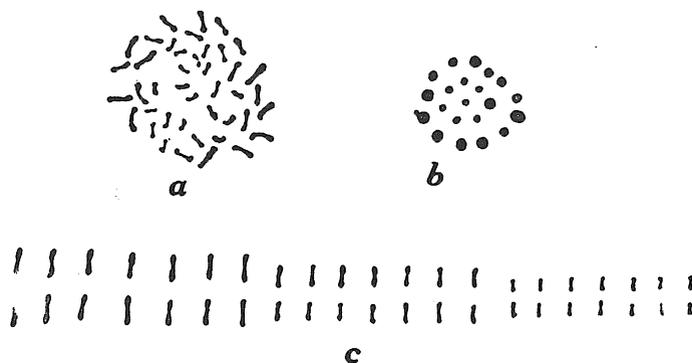


Fig. 6. *Potamogeton Friyeri* Benn.

- a. Somatic nuclear plate showing 42 chromosomes.
- b. Heterotype metaphase in polar view showing 21 bivalent chromosomes.
- c. Somatic chromosomes. (ca. $\times 2000$)

II Heteroploids.

A. Hypotetraploids.

1. *Potamogeton Kamogawaensis* Miki. $2n=27$

According to Miki, this species has taxonomically been considered to be a hybrid between *P. oxyphyllus* and *P. Miduhikimo*. This species is intermediate in size and characteristics between *P. oxyphyllus* Miq. and *P. Miduhikimo*; it differs, however, from *P. Miduhikimo* Makino in the single floating leaf and the large turion, and from *P. oxyphyllus* Miq. in the absence of the hypodermal layer, the shape of the turion and the existence of floating leaves in the inflorescence.

In this hybrid 14 chromosomes from *P. Miduhikimo* and 13 from *P. oxyphyllus* are contributed

so that 27 chromosomes would be expected as a somatic number. This was actually confirmed in root-tip cells (Fig. 7 a).

These somatic chromosomes are slender and of small size; 13 chromosomes of the 27 are longer than the remaining 14, and 5 chromosomes of the 13 long chromosomes are slightly longer than the remaining 8, and 8 chromosomes of the 14 short chromosomes are shorter than the remaining 6.

Then the karyotype of this species is as follows :

$$K(2n) = 27 = 5L_1 + 8L_2 + 6S_1 + 8S_2 \quad (\text{Fig. 7q})$$

At the first maturation division of this hybrid 0-13 bivalents chromosomes are observed. Univalents chromosomes vary from 1 to 27, depending on the number of bivalents. The frequency of the number of bivalents and univalents found in 100 dividing pollen mother cells is given in Table 1.

Table 1. Frequency of the number of bivalent and univalent found in *P. Kamogawaensis* is the count made at the metaphase.

		Number of univalent													occurrence of bivalent		
		1	3	5	7	9	11	13	15	17	19	21	23	25		27	
number of bivalents	0														6	6	
	1													5		5	
	2												6		6		
	3											5		5			
	4									9					9		
	5								6						6		
	6							6							9		
	7						13								13		
	8					7									7		
	9					6									6		
	10				8										8		
	11			9											9		
	12		6												6		
13	5													5			
occurrence of univalent		5	6	9	8	6	7	13	9	6	9	5	6	5	6	100	total number P. M. Cs. observed

The association of two component of bivalents is always loose with each other. Then the chromosome appear to be joined to a point of another, but it may not be noted as a true

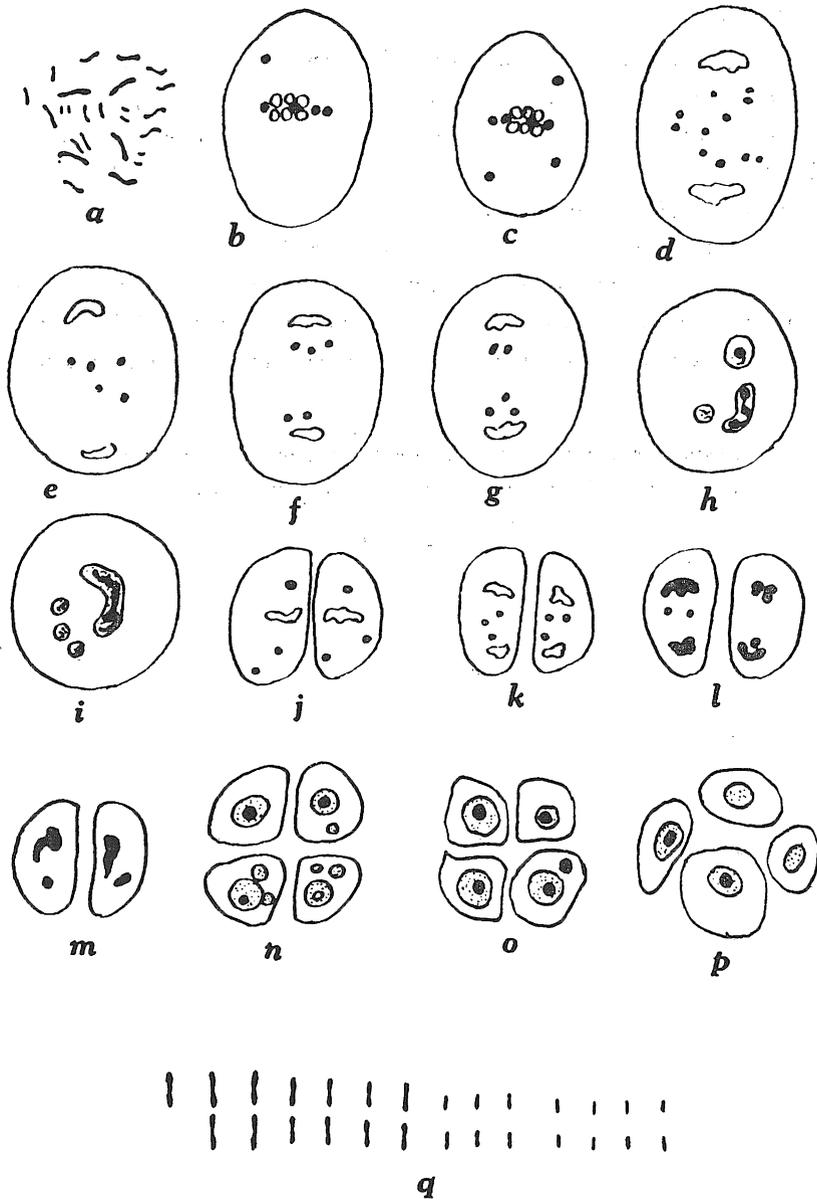


Fig. 7. *Potamogeton Kamogawaensis* Miki.

- a. Somatic nuclear plate showing 27 chromosomes.
- b. Side view of heterotype metaphase. Bivalent and univalent (shown solid black) chromosome arranged themselves at the equator.
- c. Side view of heterotype metaphase, 3 monad chromosomes scattered in the cytoplasm.
- d. Heterotype anaphase showing lagging chromosome (shown solid black)
- e. f. g. Heterotype anaphase showing lagging univalent chromosomes (solid black) in the equatorial region.
- h. i. Heterotype telophase showing nuclei of various size and shapes.
- j. Homotype metaphase in side view 3 monad chromosomes (shown solid black) are found

- scattered in the cytoplasm.
- k. Homotype anaphase showing 3 monad chromosomes lagging behind in the equatorial region.
 - l. Homotype telophase showing polynucleate sister cells
 - m. Homotype telophase showing a dwarf nucleus and a giant elongated nucleus are seen in each sister cells.
 - n. o. Irregular pollen tetrads with or without dwarf nuclei.
 - p. Irregular pollen tetrads.
 - q. Somatic chromosomes. (ca. $\times 2000$)

conjugation. At earlier stages of the metaphase of the first division all bivalents,

if present, make a normal equatorial plate. Univalents however, are more or less scattered throughout the cell. Before bivalents divide, all the univalents move to the equator and arrange themselves around bivalents (Fig. 7 b).

In a few cases some univalents are still found at some distance from that plate (Fig. 7 c). This behavior especially resembles that of the *Canina* roses reported by Täckholm (1922). In the next stage bivalents have completed their division and are moving apart on their way to the poles. At the anaphase all the divided halves of bivalents show usually homotypic splits.

The split halves of univalents separate actually and move to different poles. But in some cases split halves fail to separate and both of them, lying side by side, are distributed by chance to either of two poles (Fig. 7 d, e, f, g). The first division is, therefore, a combination of the reduction and the equatorial division. Chromosomes lagging in the cytoplasm are frequently observed in the telophase.

They are either monad or dyad chromosomes, but in some cases it is difficult to distinguish them. The monad may be regarded as the separated half of a univalent and the dyad as an entire univalent.

Most of the lagging chromosomes arrive at or near the pole where a new nucleus is just formed, but occasionally some are lost in the cytoplasm. It is sometimes observed that all or most univalents are fused to one or two large chromosome masses at the telophase of the first division (Fig. 7 h, i). These chromosome masses are later enclosed in either of two daughter cells by cytokinesis, but sometimes these masses are bridged between two daughter nuclei.

During the interkinesis the lost chromosomes or chromosome masses form additional micronuclei.

At the metaphase of the second maturation division many figures show very regular plates, but there appear occasionally aberrant chromosomes, monads and dyads, lying outside the equatorial plate (Fig. 7 j). In the succeeding stage a great number of chromosomes forming the second division plate divide longitudinally and pass to opposite poles, while a few undivided remain behind and subsequently are distributed at random to the poles. The former may be dyads and the latter monads (Fig. 7 k).

Most of the lagging chromosomes are monads, but a few are apparently dyads which may have been left outside the metaphase plate in the second division. Occasionally univalents are divided neither at the first nor at the second maturation divisions, and showing double nature, are lost in the cytoplasm of tetrad cells. In the present case some lagging chromosomes often

fail to reach the pole and are left out of the reformed daughter nuclei (Fig. 7l, m).

As in the first division most lagging chromosomes unite sometimes with each other and become chromosomes masses lying in the central portion of the cell or they extend between two poles. Although both divisions are very irregular, the resultant tetrad generally contains four cells.

Cells of a pollen tetrad have occasionally some chromatic bodies in addition to a main nucleus (Fig. 7n, o). Most of the young microspores are abnormal in shape and size.

Giant and small microspores sometimes result (Fig. 7p,).

If both the nuclear division and cytokinesis remain incomplete, a twin microspore has only one large nucleus. It is, so to speak, a kind of restitution nuclei which were observed in the meiosis of many other hybrids. It is well-known from what has been above stated that chromosome contents in the microspore differ owing to irregular meiotic division. The analogous chromosome behavior is found in many hybrids of various plants.

This species is a heteroploid plant, and is a hypoheteroploid plant.

2. *Potamogeton Fauriei* (Benn) Miki. $2n=27$

This species has taxonomically been considered by Miki, to be a hybrid between *P. oxyphyllus* and *P. monoginus*.

In this hybrid 13 chromosomes from *P. oxyphyllus* and 14 chromosomes from *P. monoginus* are contributed so that 27 chromosomes would be expected as a somatic number. This was actually confirmed in root tip cells (Fig. 8a). The somatic chromosomes are slender; 14 chromosomes of the 27 are slightly shorter than the remaining 13 and 5 chromosomes of the 13 long chromosomes are slightly longer than the remaining 8, and 6 chromosomes of the 14 short chromosomes are slightly shorter than remaining 8.

Then the karyotype of this species is as follows :

$$K(2n) = 27 = 5L_1 + 8L_2 + 8S_1 + 6S_2 \quad (\text{Fig. 8i})$$

At the first maturation division, 27 univalents are observed most frequently. Among them 14 univalent are slightly smaller than the remaining 13 and 5 of the 13 large chromosomes are slightly larger than the remaining 8, and 6 chromosomes of the 14 small chromosomes are slightly smaller than the remaining 8.

The chromosomes behavior during the meiosis of *P. M. Cs.* is quite similar to that in other plant hybrid. At the metaphase 0-7 bivalents appear. The count of bivalent was made in 100 dividing pollen mother cells is given in Table 2.

Table 2. Frequencies of the number of bivalents in *P. Fauriei* Miki counted at the first division metaphase,

Number of bivalent chromosomes	0	1	2	3	4	5	6	7	8	total
Observed frequency	74	2	6	8	2	3	3	2	0	100

Two components of a bivalent always mate loosely with each other.

They are almost always attached only to one end. At the early metaphase bivalents, if present,

are arranged on the equatorial plate, but univalents are irregularly scattered in the cytoplasm. In the succeeding stage most univalents move to the equator and arrange themselves around bivalents. Fig. 8b shows that 3 bivalents and 20 univalents are found on the division plate, and one univalent lies at a distance. Univalents remain still on that plate after divided halves of bivalents have moved apart to the poles. Homotypic splits of univalents appear at the anaphase or in some cases at more or less earlier periods of the first division. Every lagging univalent divides equationally and the halves almost invariably pass to opposite poles, though occasionally two halves lying side by side are seen to move to one pole. Some univalents may join to the group of divided halves of bivalents passing to the pole.

At the telophase 4 groups of chromosomes are often found; the outer two are the groups composed of divided halves of bivalents and the inner two those of split halves of univalents which will soon reach the poles and are included in the former. Many of the daughter nuclei, therefore, represent irregular outlines.

The second division plate is regular compared with that of the first division. The plate usually

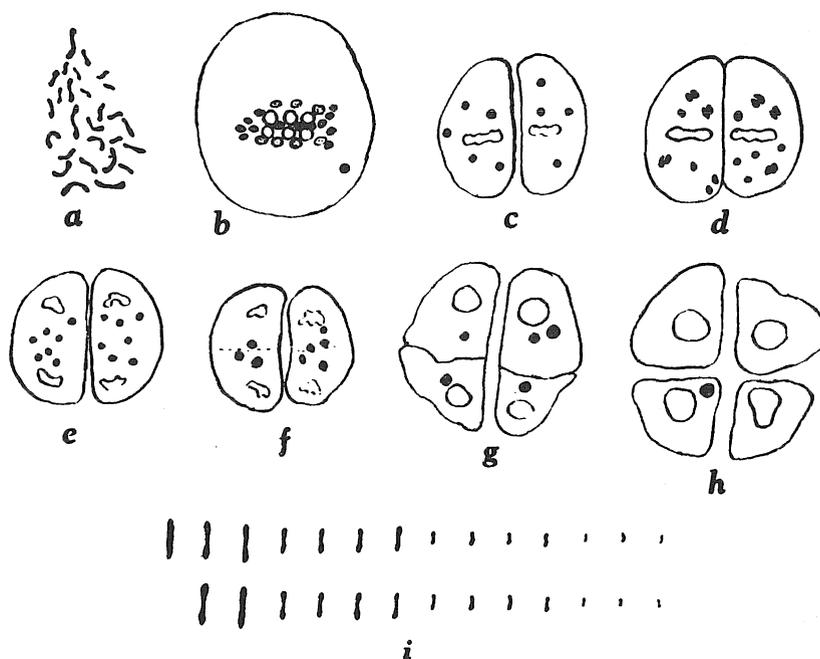


Fig. 8. *Potamogeton Fauriei* Miki.

- a. Somatic nuclear plate showing 27 chromosomes.
- b. Side view of heterotype metaphase. All chromosome except 1 univalent arrange themselves at the equator.
- c. Side view of metaphase of the second division. Some monads are scattered in the cell.
- d. Side view of metaphase of the second division showing very irregular division plates.
- e. Anaphase of the second division. Some monads are lagging in the equator.
- f. Telophase of the second division. Some chromosome are lost in the cytoplasm.
- g. A pollen tetrad with many chromatic bodies main nuclei.
- h. pollen tetrad 4 tetrad nuclei are differing in size.
- i. Somatic chromosomes. (ca. $\times 2000$)

includes the majority of chromosomes, but some apart (Fig. 8c). Fig. 8d shows very irregular plates which were sometimes found. In this case monads and dyads scattered in the whole cell are clearly seen. The number of chromosomes outside the division plate is usually 3-5, distinctly more numerous than that of chromosomes lost at the telophase in the first division. It may be stated that monads included in daughter nuclei have also a tendency to be scattered in the cytoplasm at the formation of the equatorial plate of the second division.

Dyads now divide equationally and travel to the poles, while monads do not undergo a further division and remain behind (Fig. 8e).

It can be seen that some lagging monads are much constricted.

Lagging chromosomes increase again in number compared with those at the metaphase, but are fewer than the total number of univalents rising at random to join in the reformed nucleus, though some frequently fail to join (Fig. 8f).

In a number of P. M. Cs. most lagging chromosomes are left at the central region of a cell and associate themselves in a large chromatic clump. Finally by cytokinesis this clump is constricted and separated into two portions or the entire mass is enclosed in one of two daughter cells. Thus, monads show greater irregularities in their behavior at the anaphase and the telophase of the second division than the univalents at the first division. Many supernumerary nuclei are found in young pollen tetrads, but generally four pollen grains are formed from one P. M. Ca. (Fig. 8g). Sometimes some microspores show abnormal appearance as in other plant hybrids (Fig. 8h).

3. *Potamogeton oxyphyllus* Miq. $2n=26$

This species has 26 chromosomes in the somatic cells (Fig. 9a). The same number has been reported by Harada (1942).

Of the 26 chromosomes, 12 are slightly longer than the remaining 14, and 2 chromosomes of the 12 long chromosomes are slightly longer than the remaining 10, and 6 chromosomes of the 14 short chromosomes are shorter than remaining 8. The karyotype of this species is as follows:

$$K(2n) = 26 = 2L_1 + 10L_2 + 8S_1 + 6S_2 \quad (\text{Fig. 9c})$$

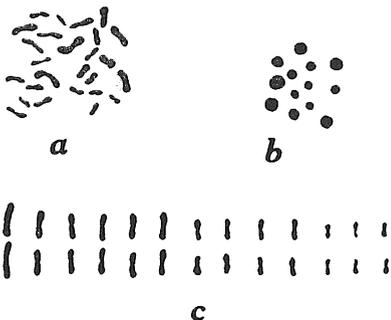


Fig. 9. *Potamogeton oxyphyllus* Miq.

- a. Somatic nuclear plate showing 26 chromosomes.
- b. Heteroty pemetaphase in polar view showing 13 bivalent chromosome.
- c. Somatic chromosomes. (ca. x200C)

In the P. M. Cs. 13 bivalent chromosomes are counted at diakinesis and metaphase in the heterotype division, 6 bivalents chromosomes of the 13 are larger than the remaining 7, and one bivalent chromosome of the 6 large bivalent chromosomes is slightly larger than the remaining 5, and 3 bivalents chromosomes of the 7 small bivalent chromosomes are smaller than the remaining 4 (Fig. 9b). The haploid chromosome number is 13 and is also found in *P. pusillus* L., *P. panormitanus* Biv. and some other species, and thus we can certainly say that in this genus there is *Potamogeton* at least two basic number of 7 and 13, the former of which we have already mentioned.

All bivalents divide almost simultaneously in the

anaphase and the divided halves move to opposite pole in a regular manner.

Two daughter nuclei enter the interkinesis condition. In the second division all chromosomes are regularly arranged on the equatorial place in the metaphase of the second division, and in the anaphase, each of the longitudinal halves of chromosomes passes to the spindle poles. The number of the pollen grains are uniform in shape and size.

4. *Potamogeton pusillus* L. $2n=26$

The somatic number of chromosomes is 26 in this specie (Fig. 10a). The same number has been reported by Palmgren (1939). Among the 26 chromosomes, 12 are slightly larger than the remaining 14, and 6 chromosomes of the 14 short chromosomes are shorter than the remaining 8.

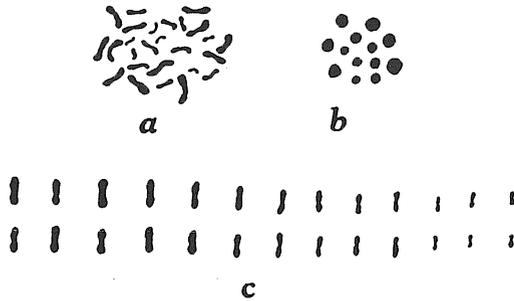


Fig. 10. *Potamogeton pusillus* L.

- a. Somatic nuclear plate showing 26 chromosomes.
- b. Heterotype metaphase in polar view showing 13 bivalent chromosomes,
- c. Somatic chromosomes. (ca. $\times 2000$)

The karyotype of this species is as follows:

$$K(2n) = 26 = 12L_1 + 8S_1 + 6S_2 \quad (\text{Fig. 10c})$$

The chromosome behavior in the P. M. Cs. is on the whole regular in this species. The number of chromosomes pairs is found in most cases to be 13 at diakinesis, and metaphase in the heterotype as well as the homotype divisions (Fig. 10b).

Among them, 6 bivalents of the 14 are larger than the remaining 7, and 3 bivalents of the 7 small bivalents are slightly smaller than the remaining 4. But this difference in size is not so distinct. In some cases, in 1 or 2 bivalents the component chromosomes are loosely associated.

5. *Potamogeton panormitanus* Biv. $2n=26$

In this species 26 chromosomes are counted in the root-tip cells (Fig. 11a).

The same number has been reported by Palmgren (1939). 12 of the 26 chromosomes are larger than the remaining 14, and 2 chromosomes of the 12 long chromosomes are slightly longer than the remaining 10, and 6 chromosomes of the 14 short chromosomes are slightly shorter than the remaining 8.

The karyotype of this species is as follows:

$$K(2n) = 26 = 2L_1 + 10L_2 + 8S_1 + 6S_2 \quad (\text{Fig. 11c})$$

In maturation division P. M. Cs. 13 bivalents are observed in diakinesis and metaphase in heterotypic division.

Among them 6 bivalents of 13 are larger than the remaining 7, and 1 bivalent of the 6 large bivalents are slightly larger than the remaining 5, and 3 bivalents of the 14 small bivalents are smaller than the remaining 4 (Fig. 11b).

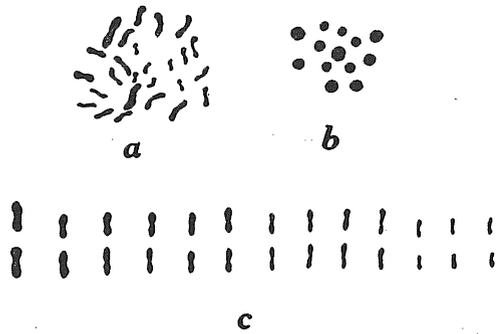


Fig. 11. *potamogeton panormitanus* Biv.
 a. Somatic nuclear plate showing 26 chromosomes.
 b. Heterotype metaphase in polar view showing 13 bivalent chromosomes.
 c. Somatic chromosomes. (ca. $\times 2000$)

All bivalents divide almost simultaneously in the anaphase, and the divided halves move to opposite poles in a regular manner. Two daughter nuclei enter the interkinesis.

The second division proceeds normally and no abnormal behavior of chromosome is observed.

The pollen tetrads, and pollen grains are uniform in shape and size.

6. *Potamogeton orientalis* Hagst $2n=26$

According to Miki, this species has taxonomically been considered to be a hybrid between *P. oxydhyllus* and *P. pusillus*. Then this hybrid 13 chromosomes from *P. oxyphyllus* and 13 chromosomes from *P. pusillus* are contributed so that 26 chromosomes would be expected as a somatic number. This was actually confirmed in root-tip cells (Fig. 12a).

Among them 12 chromosomes of 26 are longer than the remaining 14, and 2 chromosomes of 12 long chromosomes are slightly longer than the remaining 10, and 6 chromosomes of 14 short chromosomes are shorter than the remaining 8. Then the karyotype of this hybrid is as follows.

$$K(2n) = 26 = 2L_1 + 10L_2 + 8S_1 + 6S_2 \quad (\text{Fig. 12j})$$

At metaphase I. of *P. M. Cs.* 26 chromosomes are observed most frequently.

Among them 12 chromosomes are larger than the remaining 14, and 2 chromosomes of 12 large chromosomes are slightly larger than the remaining 10, and 6 chromosomes are slightly smaller than the remaining 8.

The chromosome behavior during the meiosis of *P. M. Cs.* is abnormal.

At metaphase I 1-8 bivalent chromosomes are observed. Univalent chromosomes vary from 1 to 26, depending on the number of bivalents. The frequency of the number of bivalents and univalents found in 100 dividing *P. M. Cs.* is given in Table 3.

Two components of a bivalent always mate with each other.

At the early metaphase. bivalents, if present, are arranged on the equatorial plate but univalents are irregularly scattered in the cytoplasm (Fig. 12b, c).

Table 3 Frequency of the number of bivalents and univalents found in *P. orientalis* count made at the metaphase

Number of bivalent chromosomes	0	1	2	3	4	5	6	7	8	9	10	11	Total
Observed frequency	7	10	8	12	13	17	9	21	3	0	0	0	100

In the succeeding stage most univalents move toward the equator and arranging themselves around bivalents, and remain still at that plate after divided halves of bivalents have moved apart to the spindle poles (Fig. 12d).

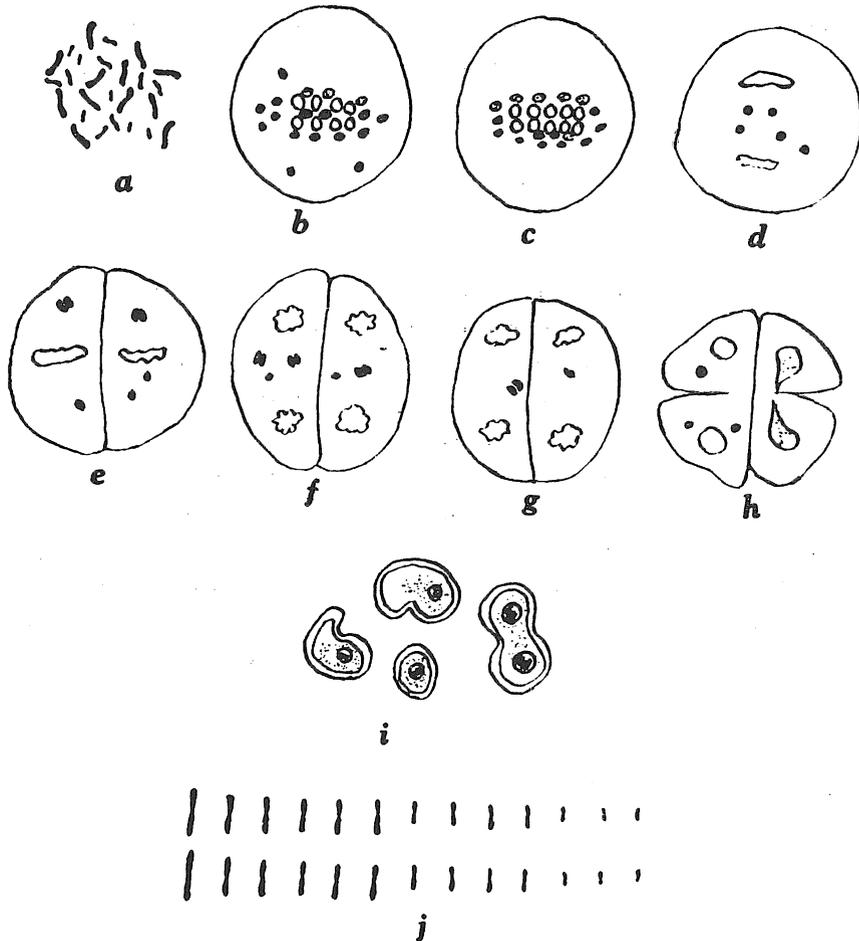


Fig. 12. *potamogeton orientalis* Hagst.

- a. Somatic nuclear plate showing 26 chromosomes.
- b. Side view of metaphase of the first division. Bivalent chromosomes arranged in equator and univalent chromosomes (show solid black) scattered in the cytoplasm.
- c. Side view of metaphase of the first division. Bivalent chromosomes arranged equator and univalent chromosomes around it.
- d. Side view of heterotype anaphase. Some univalent chromosomes lagging in equatorial region.
- e. Metaphase of the second division. Some monads and dyads are scattered in the cell.
- f. Side view of anaphase of the second division. Some monads and dyads lagging in the equatorial region.
- g. Side view of telophase of the second division.
- h. Young pollen tetrad.
- i. Abnormal pollen grains (twin, small etc.).
- j. Somatic chromosomes. (ca. $\times 2000$)

The second division plate is regular compared with that of the first division. The plate usually includes the majority of chromosomes, but some are apart from very irregular plates which were sometimes found. In this case monads and dyads scattered in the whole cell are clearly seen (Fig. 12e).

The number of chromosomes outside the division plate is usually 3-4, distinctly more numerous than that of chromosomes lost at the telophase in the first division. It may be stated that monads included in daughter nuclei have also a tendency to be scattered in the cytoplasm at the formation of the equatorial plate of the second division.

Dyads now divide equationally and move to the poles, while monads do not undergo a further division and remain behind. It can be seen that some lagging monads are very constricted. (Fig. 12f)

Lagging chromosomes increase again in number compared with those at the metaphase, but are few than the total number of univalents rising at the metaphase of the first division.

Shortly after they move at random to join to the reformed nucleus, though some frequently fail to join (Fig. 12g). In a number of P. M. Cs. most lagging chromosomes are left at the central region of a cell and associate themselves into a large chromatic clump.

Finally by cytokinesis this clump is constricted and separated into two portions or the entire mass is enclosed in one of two daughter cells (Fig. 12h). Many supernumerary nuclei are found in young pollen tetrads, but generally four pollen grain are formed from one pollen mother cells. Sometimes some microspores show abnormal appearance (Fig. 12i), such figures may have resulted from an interkinesis cell which cytokinesis has incompletely took place for prevention of a chromosome mass lying at the central region of the cell.

B. Hypooctaploids

1. *Potageton malaiianus* Miq. $2n=52$

The somatic chromosome number of this species was determined to be 52 (Fig. 12a). The same number has been reported by Harada (1942).

Among them 24 are longer than the remaining 28, and 12 chromosomes of the 24 long chromosomes are longer than the remaining 12, and 14 chromosomes of the 28 short chromosomes are shorter than the remaining 14.

Then the karyotype of this species is as follows:

$$K(2n) = 52 = 12L_1 + 12L_2 + 14S_1 + 14S_2 \quad (\text{Fig. 13c})$$

At metaphase I 26 bivalents chromosomes are observed among them 12 bivalents of 26 are larger than the remaining 14, and 6 bivalent of the 12 large bivalents are larger than the remaining 6, and 7 bivalents of the 14 small bivalents are smaller than the remaining 7 (Fig. 13b). The behavior of chromosomes in disjunction is regular in general.

The maturation divisions are quite regular, and no significant irregularities have been found. *Forma subfluitans* Makino is the same species with *P. malaiianus* Miq..

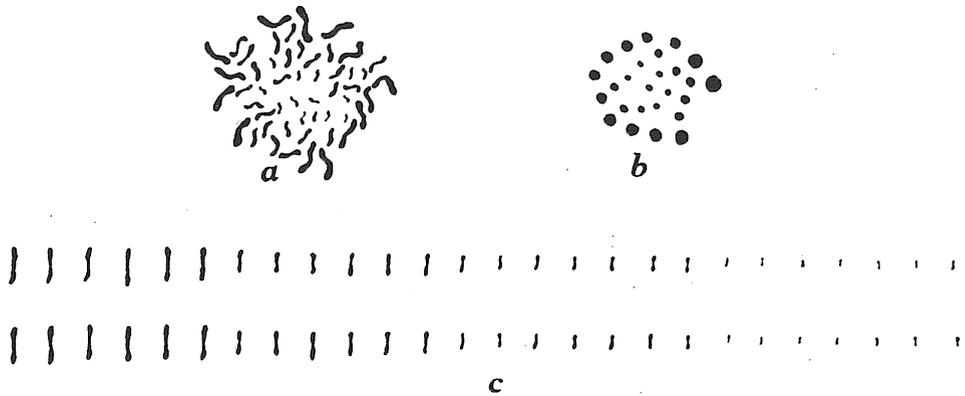


Fig. 13. *Potamogeton malaianus* Miq.
 a. Somatic nuclear plate showing 52 chromosomes.
 b. Heterotype metaphase in polar view showing 26 bivalent chromosomes.
 c. Somatic chromosomes. (ca. $\times 2000$)

2. *Potamogeton dentatus* Hagst. $2n=52$

52 somatic chromosomes number are counted in root-tip cells (Fig. 14 a). The same number has been reported by Harada (1942).

The karyotype of this species is quite the same as that of *P. malaianus*.

$$K(2n) = 52 = 12L_1 + 12L_2 + 14S_1 + 14S_2 \quad (\text{Fig. 14c})$$

In diakinesis and metaphase I the size and shape of chromosomes is quite the same as that of *P. malaianus* (Fig. 14b).

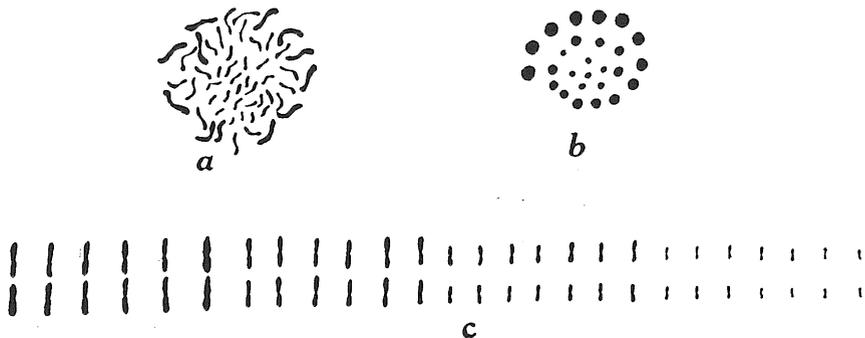


Fig. 14. *Potamogeton dentatus* Hagst.
 a. Somatic nuclear plate showing 52 chromosomes.
 b. Heterotype metaphase in polar view showing 26 bivalent chromosomes.
 c. Somatic chromosomes. (ca. $\times 2000$)

3. *Potamogeton alpinus* Bolb. $2n=52$

52 somatic number of chromosomes are counted in root-tip cells (Fig. 15a). Among them 24 are longer than the remaining 28.

Then the karyotype of this species is as follows :

$$K(2n)=52=24L_1+28S_1 \quad (\text{Fig. 15c})$$

The chromosome behavior in the maturation division of the P. M. Cs. is, on the whole, regular in this species. The bivalent chromosomes are found in most cases to be 26 at diakinesis and metaphase in the heterotype (Fig. 15b).

The bivalents can be divided into two classes according to their size; the chromosomes of the first class which are 12 in number are larger than the 14 chromosomes of the other.

All the chromosomes are round in shape. The division figures are, practically speaking, all regular both in the heterotype and homotype divisions.

The pollen grains are mostly normal in appearance.

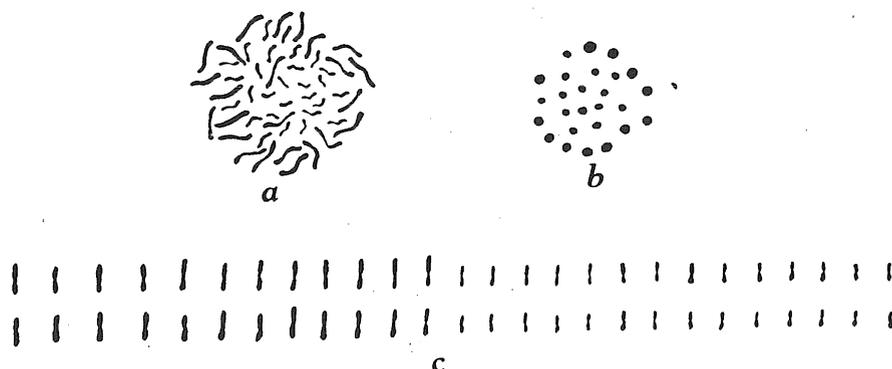


Fig. 15. *Potamogeton alpinus* Balb.

- a. Somatic nuclear plate showing 52 chromosomes.
- b. Heterotype metaphase in polar view showing 26 bivalent chromosomes.
- c. Somatic chromosomes. (ca. $\times 2000$)

4. *Potamogeton perfoliatus* L. $2n=52$

This species shows 52 chromosomes in the root-tip cells (Fig. 16a). The same number has been reported by Wisniewska (1931).

24 chromosomes of the 52 are slightly longer than the remaining 28.

Then the karyotype of this species is as follows:

$$K(2n)=52=24L_1+28S_1 \quad (\text{Fig. 16c})$$

In maturation division are found 26 bivalents in the heterotype metaphase (Fig. 16b). The size relation among those bivalents is recognizable as in the case of *P. alpinus*.

12 bivalents of the 26 are larger than the remaining 14. Nothing abnormal is found in the chromosome behavior in the division.

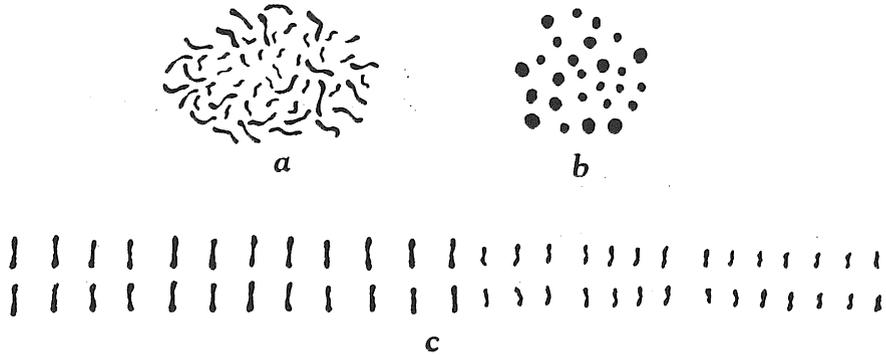


Fig. 16. *Potamogeton perfoliatus* L.

- a. Somatic nuclear plate showing 52 chromosomes.
- b. Heterotype metaphase in polar view showing 26 bivalent chromosomes.
- c. Somatic chromosomes. (ca. $\times 2000$)

5. *Potamogeton crispus* L. $2n=52$

In this species 52 chromosomes are found in the root-tip cells (Fig. 17a) and the same number has been reported by Palmgren. (1939). Of these chromosomes 24 are longer than the remaining 28, and 14 chromosomes of 28 short chromosomes are shorter than the remaining 14.

Then the karyotype of this species is as follows:

$$K(2n)=52=24L_1+14S_1+14S_2 \quad (\text{Fig. 17c})$$

The chromosome behavior in the maturation division of the P. M. Cs. is on the whole regular in this species. The number of chromosome pairs is found in most cases to be 26 at diakinesis and metaphase in the heterotype as well as the homotype division (Fig. 17b). 12 of 26 bivalents are larger than the remaining 14 and 7 of 14 small bivalents are smaller than the remaining 7. The members of the pollen tetrad and pollen grains are uniform in shape and size. No significant irregularities have been found.

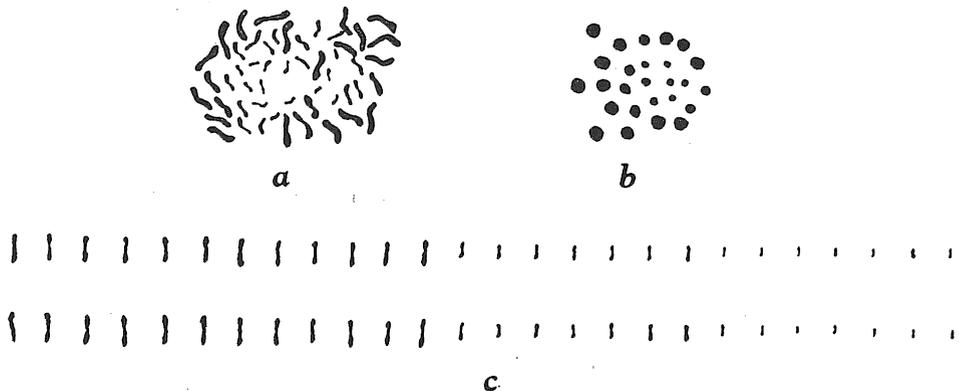


Fig. 17. *Potamogeton crispus* L.

- a. Somatic nuclear plate showing 52 chromosomes.
- b. Heterotype metaphase in polar view showing 26 bivalent chromosomes.
- c. Somatic chromosomes. (ca. $\times 2000$)

6. *Potamogeton praelongus* wulf. $2n=52$

The somatic number of chromosomes is 52 counted in root-tip cells (Fig. 18 a). The same number has been reported by Palmgren (1939). 24 chromosomes of 52 are longer than the remaining 28.

The Karyotype of this species is as follows:

$$K(2n) = 52 = 24L_1 + 28S_1 \quad (\text{Fig. 18d})$$

The chromosome behavior in meiosis of P. M. Cs. is on the whole regular in this species. The bivalent chromosomes is found in most cases to be 26 at diakinesis and metaphase I (Fig. 18b), 12 of 26 bivalents are slightly larger than the remaining 14. In some cases, in 1 or 2 bivalents the component chromosomes are very loosely associated, or even separated from each other, so that more than 26 chromosomes elements are counted in the heterotype nuclear plate.

In the second division the metaphase plates are very regular (Fig. 18 c). The two plates are disposed at right angles or parallel to each other.

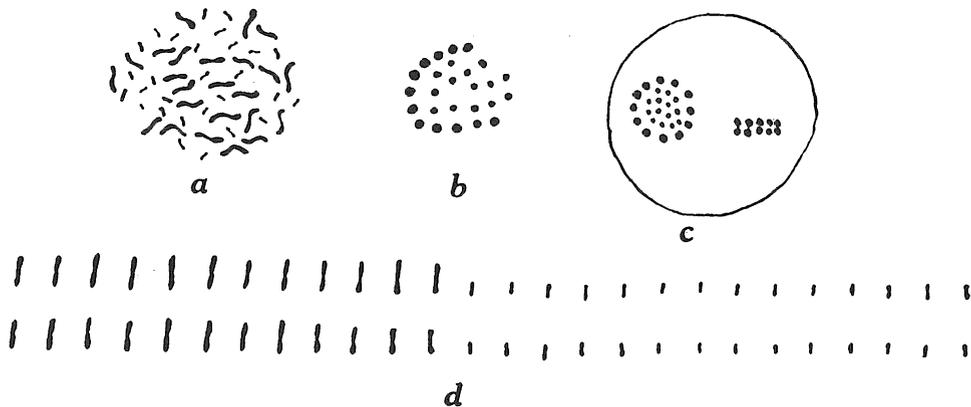


Fig. 18. *Potamogeton praelongus* Wulf.

- a. Somatic nuclear plate showing 52 chromosomes.
- b. Heterotype metaphase in polar view showing 26 bivalent chromosomes.
- c. Metaphse of the second division.
- d. Somatic chromosomes. (ca. $\times 2000$)

7. *Potamogeton Maackianus* Benn. $2n=52$

In the metaphase of somatic mitosis we counted 52 chromosomes (Fig. 19 a). The same number has been reported by Harada (1942).

In those chromosomes 24 chromosomes of 52 are slightly longer than the remaining 28.

The karyotype of this species is as follows :

$$K(2n) = 52 = 24L_1 + 28S_1 \quad (\text{Fig. 19c})$$

In maturation division of P. M. Cs., 26 bivalents are observed in diakinesis and metaphase in the heterotypic division. Among them 12 are larger than the remaining 14 (Fig. 19 b). All bivalents divide almost simultaneously in the anaphase, and the divided halves move to opposite poles in a regular manner. Two daughter nuclei enter the interkinesis.

In the second division all chromosomes are regularly arranged on the equatorial plane in the metaphase of the second division, and in the anaphase each of the longitudinal halves of chromosomes, they pass to the spindle poles. The members of the pollen tetrad and pollen grains are uniform in shape and size.

P. tenuifolius Camus is a same species with *P. Maackianus* Benn..

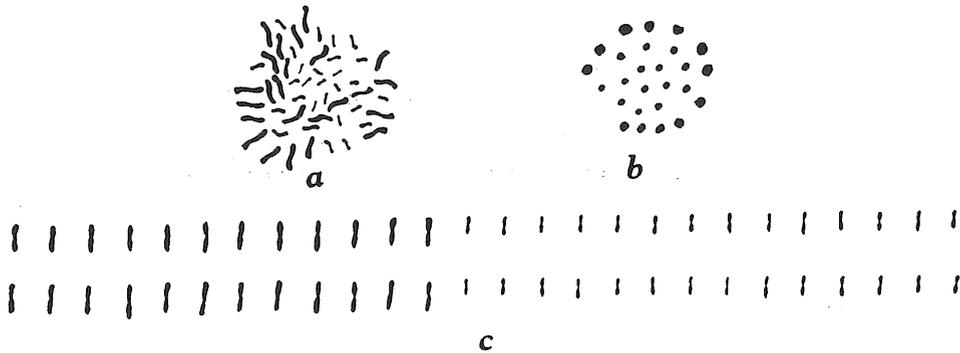


Fig. 19. *Potamogeton Maackianus* Benn.

- a. Somatic nuclear plate showing 52 chromosomes.
- b. Heterotype metaphase in polar view showing 26 bivalent chromosomes.
- c. Somatic chromosomes. (ca. $\times 2000$)

8. *Potamogeton natans* L. $2n=52$

In this species somatic number of chromosomes are 52 counted in root-tip cells (Fig. 20 a). The same number has been reported by Palmgen (1939).

The karyotype of this species is quite the same as that of *P. Maackianus*

$$K(2n) = 52 = 24L_1 + 28S_1 \quad (\text{Fig. 20c})$$

In the maturation division of *P. M.* Cs., the size and shape of chromosomes is quite the same as that of *P. Maackianus* (Fig. 20b).

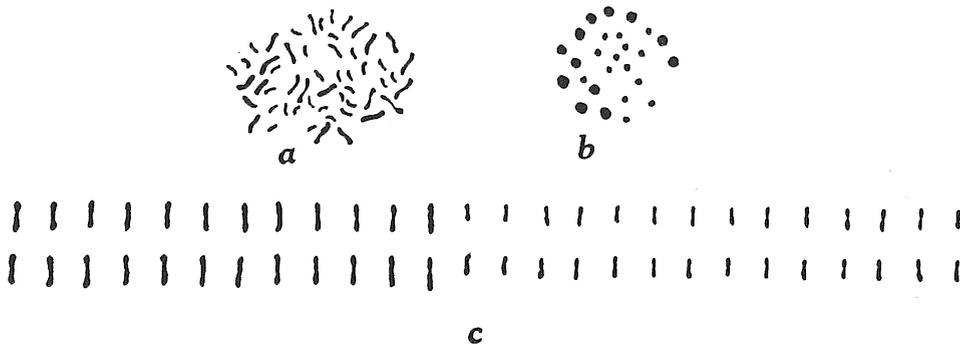


Fig. 20. *Potamogeton natans* L.

- a. Somatic nuclear plate showing 52 chromosomes.
- b. Heterotype metaphase in polar view showing 26 bivalent chromosomes.
- c. Somatic chromosomes. (ca. $\times 2000$)

9. *Potamogeton distinctus* Benn. $2n=52$

The somatic number of chromosomes is 52 counted in root-tip cells (Fig 21 a). The same number has been reported by Harada (1942).

38 chromosomes of 52 are slightly longer than the remaining 14, and 2 chromosomes of 38 long chromosomes are slightly longer than the remaining 36.

Then the karyotype of this species is as follows:

$$K(2n)=52=2L_1+36L_2+14S_1 \quad (\text{Fig. 21c})$$

In P. M. Cs., 26 bivalents chromosomes are counted at diakinesis and metaphase I and 26 univalents at metaphase in the homotype division (Fig. 21b). All the chromosomes are round in shape. The division figures are practically speaking all regular both in the heterotype and homotype divisions. The pollen grains are mostly normal in appearance.

P. Teperi Benn., *P. Francketii* Benn. et Baaøe, *P. longipetiolatus* Camus are same species with *P. distinctus* Benn..

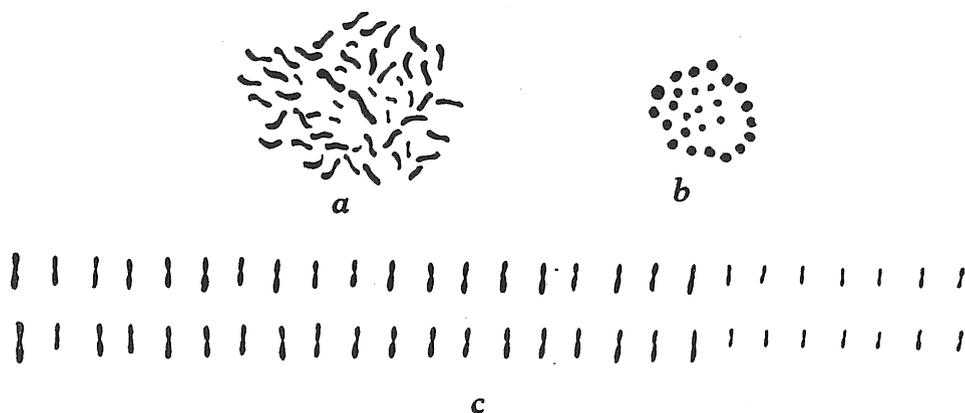


Fig. 21. *Potamogeton distinctus* Benn.

- a. Somatic nuclear plate showing 52 chromosomes.
- b. Heterotype metaphase in polar view showing 26 bivalent chromosomes.
- c. Somatic chromosomes. (ca \times 2000)

10. *Potamogeton biwaensis* Miki. $2n=52$

This species has taxonomically been considered by Miki to be a hybrid between *P. maackianus* Benn. and *P. nipponicus* Makino.

Observations in root-tip cells (Fig. 22a), as in the case of the *P. maackianus* Benn. of the 52 chromosomes 24 are slightly longer than the remaining 28. The same number has been reported by Harada (1942).

The karyotype of this species is as follows:

$$K(2n)=52=24L_1+28S_1 \quad (\text{Fig. 22c})$$

In P. M. Cs. 26 bivalents chromosomes are counted a diakinesis and metaphase I and 26 bivalents at metaphase II (Fig. 22b).

The division figures are practically speaking, all regular both in the heterotype and homotype divisions.

The pollen grains are mostly normal in appearance.

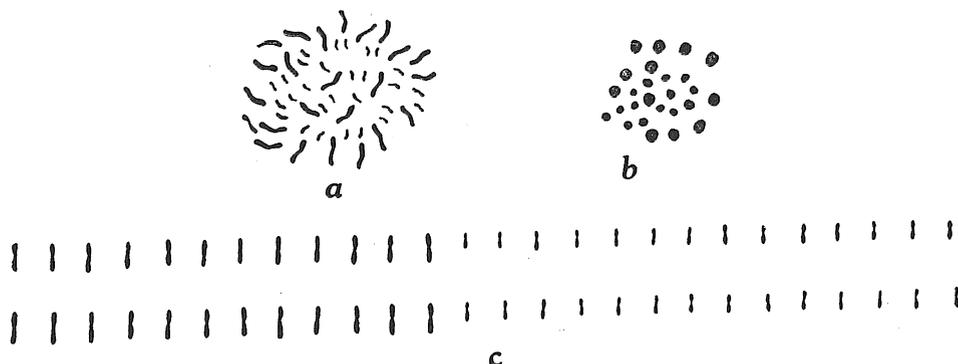


Fig. 22. *Potamogeton biwaensis* Miki.

- a. Somatic nuclear plate showing 52 chromosomes.
- b. Heterotype metaphase in polar view showing 26 bivalent chromosomes.
- c. Somatic chromosomes. (ca. $\times 2000$)

11. *Potamogeton malaianoides* Miki $2n=52$

This species has taxonomically been considered by Miki to be a hybrid between *P. distinctus* Benn. and *P. malaianus* Miq.

The somatic chromosome number is 52 in this species (Fig. 23a).

24 chromosomes of 52 are longer than the remaining 28, and 14 chromosomes of 24 short chromosomes are slightly shorter than the remaining 14.

Then the karyotype of this hybrid is as follows:

$$K(2n)=52=24L_1+14S_1+14S_2 \quad (\text{Fig. 23c})$$

The behavior of chromosomes in the P. M. Cs. is regular in this hybrid. The number of chromosome pair is found in most cases to be 26 at diakinesis and metaphases in the heterotype as well as in the homotype division (Fig. 23b).

Among 26 bivalents, 14 are smaller than the remaining 12, and 7 of 14 small bivalents are smaller than the remaining 7, but this difference in size is not so distinct. In some cases, in 1 or 2 bivalents the component chromosomes are very loosely associated, or even quite separated from each other, so that more than 26 chromosomal elements are counted in heterotype nuclear plate.

In the second division the metaphase plate are very regular.

The two plates are disposed at right angles or parallel to each other.

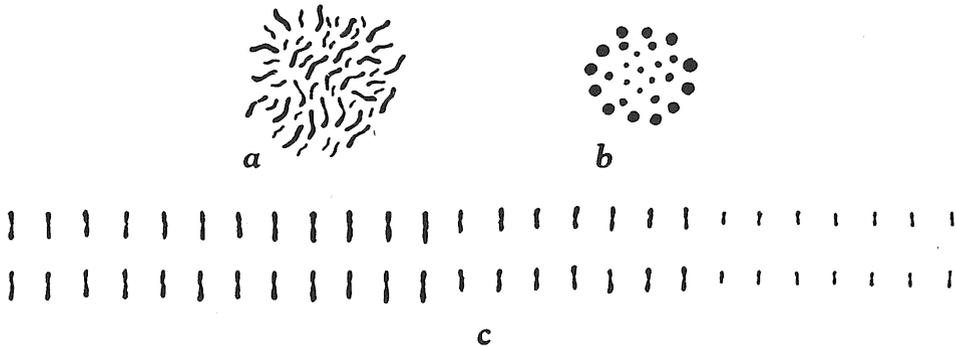


Fig. 23. *Potamogeton malainoides* Miki.

- a. Somatic nuclear plate showing 52 chromosomes.
- b. Heterotype metaphase in polar view showing 26 bivalent chromosomes.
- c. Somatic chromosomes. (ca.×2000)

12. *Potamogeton anguillanus* Koidz. $2n=52$

This species has taxonomically been considered by Miki to be a hybrid. The somatic number of chromosomes 52 are counted in root-tip cells (Fig. 24a). Among them 24 chromosomes of the 52 are longer than the remaining 28.

The karyotype of this species is as follows :

$$K(2n) = 52 = 24L_1 + 28S_1 \quad (\text{Fig. 24c})$$

In maturation division of P. M. Cs. 26 bivalent chromosomes are counted at diakinesis and metaphase in the heterotype division and 26 univalents metaphase in the homotype division (Fig. 24b).

Among them 12 bivalents of the 26 are larger than the remaining 14.

The division figures are all regular both in the heterotype and homotype divisions. The pollen grains are mostly normal in appearance.

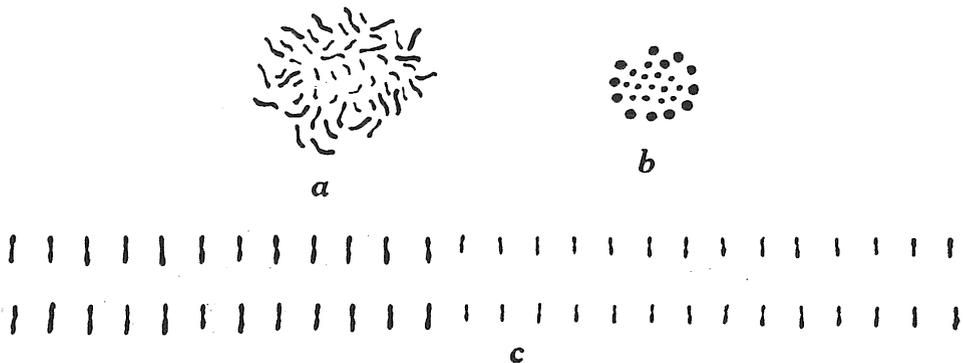


Fig. 24. *Potamogeton anguillanus* Koidz.

- a. Somatic nuclear plate showing 52 chromosomes.
- b. Heterotype metaphase in polar view showing 26 bivalent chromosomes.
- c. Somatic chromosomes. (ca.×2000)

13. *Potamogeton nipponicus* Mak. $2n=52$

This species has taxonomically been considered by Miki to be a hybrid. The somatic number of chromosomes, 52 is counted in root-tip cells (Fig. 25a). The same number has been reported by Harada (1942).

Of these chromosomes 24 are longer than the remaining 28, and 12 chromosomes of the 24 long chromosomes are slightly longer than the remaining 12, and 14 chromosomes of the 28 short chromosomes are shorter than the remaining 14.

Then the karyotype of this species is as follows:

$$K(2n)=52=12L_1+12L_2+14S_1+14S_2 \quad (\text{Fig. 25c})$$

In diakinesis and metaphase in the heterotype division, 26 bivalent chromosomes are observed (Fig. 25b). In these bivalents chromosomes 12 bivalents of the 26 are larger than the remaining 14 and 6 bivalents of the 12 large bivalents are slightly larger than the remaining 6, and 7 bivalents of the 14 small bivalents chromosomes are smaller than the remaining 7.

All bivalents divide almost simultaneously in the anaphase, and the divided halves move to opposite pole in a regular manner. Two daughter nuclei enter the interkinesis condition. All chromosomes are regularly arranged on the equatorial plane in the metaphase of the second division, and in the anaphase each of the longitudinal halves of chromosomes passes to the Pole. the members of the pollen tetrad and pollen grains are uniform in shape and size. The maturation division is quite regular and no significant irregularities have been found.

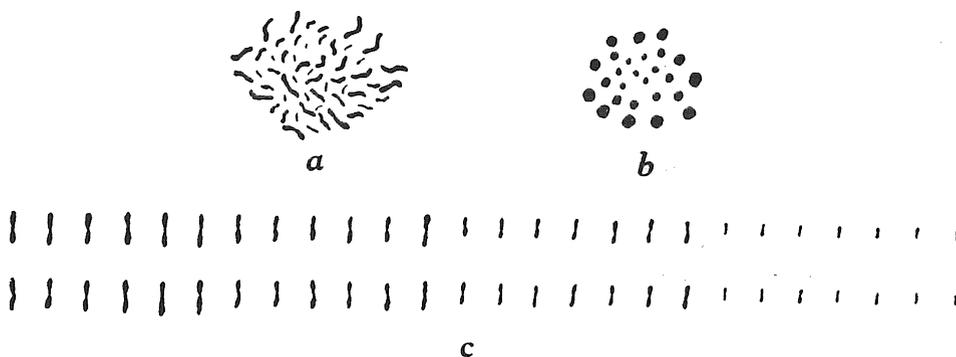


Fig. 25. *Potamogeton nipponicus* Mak.

- a. Somatic nuclear plate showing 52 chromosomes.
- b. Heterotype metaphase in polar view showing 26 bivalent chromosomes.
- c. Somatic chromosomes. (ca. $\times 2000$)

C. Hypododecaploids

1. *Potamogeton pectinatus* L. $2n=78$

The somatic number of chromosomes is 78 in root-tip cells (Fig. 26 a) and the same number has been reported by Harada, 36 chromosomes of 78 are slightly longer than the remaining 42.

Then the karyotype of this species is as follows:

$$K(2n) = 78 = 36L_1 + 42S_1 \quad (\text{Fig. 26c})$$

At metaphase the chromosomes typically lie nearly in a single plane at the equator of the spindle, forming the equatorial plate. 39 bivalent chromosomes are counted at diakinesis and metaphase I (Fig. 26b)

18 bivalents chromosomes of 39 are slightly larger than the remaining 21.

All the chromosomes are round in shape. The division figures all regular both in the heterotype and homotype divisions.

The pollen grains are mostly normal in appearance.

P. filiformis Pers., *P. vaginatus* Turcz., *P. flabellatus* Bab. are the same species as *P. pectinatus* L..

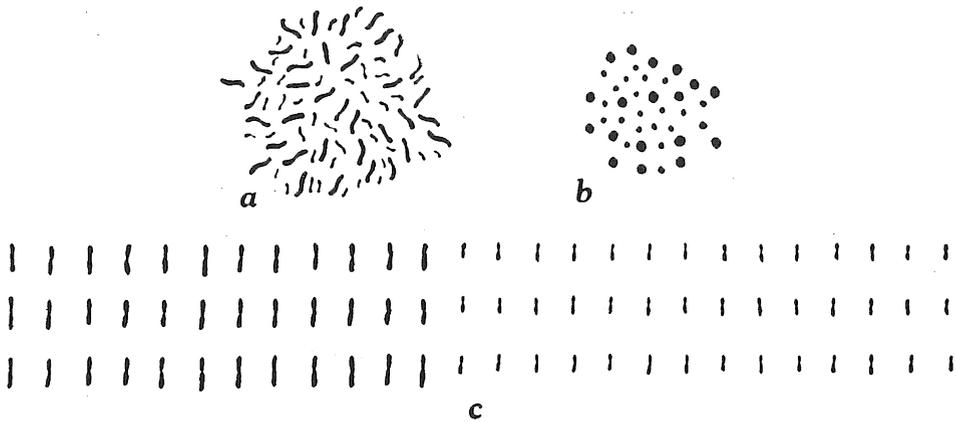


Fig. 26. *Potamogeton pectinatus* L.

- a. Somatic nuclear plate showing 78 chromosomes.
- b. Heterotype metaphase in polar view showing 39 bivalent chromosomes.
- c. Somatic chromosomes. (ca. $\times 2000$)

DISCUSSION

I. Basic number of chromosome in the genus *Potamogeton*.

In this genus several different number of chromosomes have been reported by wiegand, (1899) wisniewska, (1931) Kuleszanka, (1934) Palmgren, (1939) Takusagawa, (1939) Harada, (1942) Takusagawa, (1950) and Harada (1956).

So far as the results of my investigation are concernd, there are two basic number of chromosomes in this genus, namely 7 and 13.

The species or hybrids in which the chromosome number is a multiple of 7 are : *P. monoginus*, *P. cristatus*, *P. vaseyi*, *P. numasakinus*, and *P. Friyeri*. *P. monoginus*, *P. cristatus*, *P. vaseyi*, *P. numasakianus* are tetraploid ($2n=28$) and *P. apertus* and *P. Friyeri* are hexaploid ($2n=42$), The species or hybrids in which the chromosome number is a multiple of 13 are *P. oxyphyllus*.

P. panormitanus, *P. pusillus*, *P. orientalis*, *P. malaianus*, *P. dentatus*, *P. nipponicus*, *P. perfoliatus*, *P. crispus*, *P. praelongus*, *P. maackianus*, *P. biwaensis*, *P. alpinus*, *P. distinctus*, *P. malainoides*, *P. natans*, *P. pectinatus* and *P. anguillanus*.

P. oxyphyllus, *P. panormitanus*, *P. pusillus*, *P. orientalis* are hypotetraploid ($2n=26$). *P. malaianus*, *P. dentatus*, *P. nipponicus*, *P. perfoliatus*, *P. crispus*, *P. praelongus*, *P. maackianus*, *P. biwaensis*, *P. alpinus*, *P. distinctus*, *P. malainoides*, *P. natans* and *P. anguillanus* are hypooctaploid ($2n=52$). *P. pectinatus* is hypododecaploid ($2n=78$).

P. kamogawaensis and *P. Fauriei* are hypotetraploid ($2n=27$).

P. monoginus, *P. cristatus* and *P. vaseyi* have 14 bivalents chromosomes in heterotypic metaphase of maturation division in P. M. Cs. and 28 chromosomes in root-tip cells.

P. oxyphyllus, *P. panormitanus* and *P. pusillus* have 13 bivalents chromosomes in heterotypic metaphase of maturation division in P. M. Cs, and 26 chromosomes in root-tip cells. The 28 chromosomes group as compared with the 26 chromosomes group, as we have described for these species in the forgoing chapter, the 28 chromosome group has 14 large chromosomes and 14 small chromosomes. The 26 chromosomes group have 12 large chromosomes and 14 small chromosomes, and by the behavior of the chromosomes in meiosis can reveal that one pair of 14 large chromosomes are lost and 26 chromosomes group are newly composed. In *P. oxyphyllus*, *P. pusillus* and *P. panormitanus* all the chromosome numbers hither to found in these species are 26. This is a heteroploid number, the somatic number of chromosomes being 26. Observation of the meiotic division in pollen mother cells have proved that the number 13 is haploid number.

Investigation into the behavior of the chromosomes in meiosis can reveal that they are 13 and hence the plant is hypotetraploid.

In *P. malaianus*, *P. dentatus*, *P. nipponicus*, *P. perfoliatus*, *P. crispus*, *P. praelongus*, *P. maackianus*, *P. biwaensis*, *P. distinctus*, *P. natans*, *P. alpinus*, *P. malainoides*, on the other hand, all the chromosome number hither to found in various species are 26, the somatic number of chromosomes being 52. Observations of the meiotic division in P. M. Cs. have proved that the number 26 is the haploid number. Then these species are hypooctaploid. Harada (1942) has reported other chromosome number which I have not found in any of the species and hybrid at my disposal. According to him, in *P. Fauriei* the somatic number is 29, and in *P. Kamogawaensis* it is 42, and in *P. Friyeri* it is 52. According to the result I have obtained *P. Fauriei* and *P. Kamogawaensis* are 27 in somatic number, and *P. Friyeri* is 42 in somatic number.

It may be observed that the *P. Friyeri* has interspecific polyploid.

And Harada adds a new basic number 14 to those given above. The number 14 as a basic number may put the conclusion that the number 7 is without doubt the basic number in *Potamogeton*. If, then, the latter conclusion is wrong and the former number is correct, in those species of *P. apertus* and *P. Friyeri* somatic chromosomes number are 42. In the metaphase in the heterotype division, there are found 21 bivalents, then these are hexaploid. This fact substantiates the view that number 7 is at least a basic number in *Potamogeton*.

According to Wiegand (1899) in *P. foliosus* in diakinesis and metaphase in the heterotype division 7 bivalent chromosomes are observed. The fact substantiates the view that the number 7 is at least

a basic number in *Potamogeton*. We have, moreover, other facts which substantiate the view that the number 7 is at least the basic number in *P. cristatus*, *P. monoginus*, *P. vaseyi* and other speies. 7 bivalents of the 14 are large and 7 small. In *P. oxyphyllus*, *P. panormitanus*, *P. pusillus*, and othe species. 6 bivalents of the 14 are large and 7 small. If in these cases the basic number is 13 or 14, these sete of chromosomes must consist of similar sets each. This is assumable in both cases. In p. *Fauriei*, a chromosome hybrid between 13 and 14, the two groups of chromosomes are shown to represent independent genomes. No affinity is shown between the chromosomes of 13 and those of the 14. They are found to be all univalent in the heterotype metaphase, and neither of the two chromosomes is a mating pair in *P. Kamogawaensis*, *P. oxyphyllus-vaseyi* hybrid, and the 14 of the *P. vaseyi* chromosomes also remain as univalents having no mate at all in the heterotype division. These facts show that the 14 univalent chromosomes from the parent *P. vaseyi* have no mating chromosomes not only in the group of chromosomes from the other parent, but also among chromosomes in their own group. To put it into other words, this group of the 14 chromosomes in *P. vaseyi* is a diploid set which composes two genomes. The number 7 must, therefore, be basic number of this species.

The general conclutions which we can draw at present are, therefore, as follows;

1. In the present state of knowledge we must admit that in the genus *Potamogeton* there are two basic numbers 7 and 13.
2. The 13 chromosoomes group is derived from the 7 chrom some group.
3. It must be left for futuer researches to decide whether the number 14 represents one of the basic numbers in *Potamogeton* as considered by Harada.

II. Origin of polyploidy in the genus *Potamogeton*.

The problem will be discussed as follows as the result of investigation.

1. An essential phenomenon for the growth of polyploid plants is that it is precede by the arising of gametes which carry a corresponding number of the haploid sets of chromsomes. It has been shown by many investigations that in haploid and triploid as well as in many hybrids, an irregular distribution of chromosomes cmmonly take place in the meiosis. This kind of irregularity is also of cmmmon occurrence in *Potamogeton*, especially in its heteroploid forms. The irrsgularity appears to give rise to the formation of gametes which carry aberrant number of chromosomes, and it appears that it plays a great role in the arising of individuals with different numbers of chromosomes.

But we have facts that go to prove that most of the tetrad cells, produced through such an irregular meiotic process can not function owing to illegitimate combinations of chromosomes. In the haploid mutant of *Datura*; Belling (1926) has found that in meiosis the distribution of the 12 chromosomes to the poles takes place by chance, resulting in pairs of chromosome groups: 11 : 1, 10 : 2, 9 : 3, 6 : 6, and according to Blakeslee and Cartledge (1926, 1927) 80% of the pollen grains are empty in this haploid.

In a haploid nutant of *Oenothera franciscana* Davis and Kulkarni (1930) have found that such pollen grains amount to 60-90%. Examples are also found in heteroploid species of

Potamogeton.

In these plants, 60–90% of the pollen grains are empty, and in the extreme case which is exemplified by *P. Kamogawaensis* almost all of them are empty.

2. Another phenomenon for the arising, of polyploid individuals is that it is preceded by the arising of diploid pollen formation.

According to Belling (1926), in *Datura*, diploid pollen occurs frequently in the haploid, much more frequently than in the diploid or even in the haploid, much more frequently than in the diploid or even in the triploid two groups of 12 unsplit chromosomes each are formed at the anaphase. There is no further division and only two pollen grains result. In non-reduction in haploids the chromosomes at the first metaphase become 4-lobed and show the split while still in the equatorial plate. The first maturation division is entirely omitted in this haploid, and therefore formation of dyads occurs instead of tetrads.

A similar behavior has also been observed by Karpeckenko (1927, 1928) in *Raphanus-Brassica* hybrid.

This case was not observed in *Potamogeton*.

3. Suppression or retardation of the meiosis may be mentioned as a noteworthy phenomenon which is one of the causes of the formation of diploid gametes.

This phenomenon has been observed in several plants and in *potamogeton*, too. Rosenberg (1917, 1926–27) has pointed out the following two type of the peculiarity which he found in the *Euhieracium* species.

(a) At the heterotype metaphase or anaphase the process of division does not proceed further but is arrested by the premature homotype division, whereby the restitution nucleus—as it is called by him—is formed.

(b) In the second type, the prophase nucleus in the heterotype division contracts markedly together with the cytoplasm surrounding it, this being followed by a stage in which the nucleus shows the characteristic feature in the interkinesis that every chromosome is split longitudinally.

In both types the final results are the same, this being the formation of the pollen dyad.

Rosenberg regards this peculiarity as an important process in the doubling of the chromosome number in organisms.

4. The fusion of the homotype spindles is also an important phenomenon in regard to the doubling of the chromosome numbers in gametes. This case has been observed by several investigators in many plants, such as Ljungdahl (1922) in *Papaver*, Goodspeed (1923) in *Nicotiana*, Afzelius (1924) in *Senecio*, Matsuda (1927) in *Petunia*, Karpechenko (1927) in *Raphanus x Brassica*, Fukuda (1927) in the *Potato*, Hakanson (1929a) in *Salix*, Müntzing (1930) in *Galeopsis*, this mode of chromosome doubling is not found in *Potamogeton*.

5. In many hybrid plants it is often observed that the division of unpaired, univalent chromosomes, some or all, is equational in either one of the two divisions, heterotype or homotype, the distribution is merely random. This fact has been observed, by Clausen (1926–27) in *viola hyperchromatica*, by Sax (1931) in *Rhoeo discolor*.

Brieger and Darlington regard this type of division as one of the probable processes of polyploid formation. This type of chromosome doubling not actually been observed in *Potamogeton*.

6. Several plants are known have cells carrying the di-diploid number of chromosomes in somatic tissues.

This case has been observed by several authors in several plants, such as De Litardiere (1923, 1924) in *Spinacia* and *Cannabis*, Chimpu (1929) in *Acacia*, Lesley (1925), Lindstrom and Koos (1931) in the tomato, Clausen (1930) in *Viola* and some others.

Experimentally Jörgensen (1928) and Lindstrom and Koos (1931) have obtained diploid and tetraploid plants from the haploid and diploid ones respectively by the method of decapitation of young sprouts. Jörgensen says: "The majority of polyploid forms, in my opinion owe their origin to the doubling process ("endo-duplication") in the somatic tissue. Considering the widespread occurrence of binucleate cells in the soma and continuous somatic development of most plants, it is only natural that endo-duplication must be an important factor in the formation of polyploid plants".

This phenomenon was not observed in *Potamogeton*.

7. In some plants there are sometimes found P. M. Cs. containing two nuclei instead of one, a fact which may be regarded as another process of chromosome doubling in gametes. As pointed out by Karpechenko (1924, 1927, 1928), a premeiotic nuclear division must have taken place without being accompanied by cell division, resulting in the formation of binucleate pollen mother cells. If these nuclei fuse together during the process of meiotic division, giant diploid pollen grains will be produced. This fact has been observed by Gates and Rees (1921) in *Lactuca*, by Inariyama (1929) in *Iris*, by Randolph and McClintock (1926) in *Zea* and by Ichijima (1926, 1930) in *Fragaria*. This case is not found in *Potamogeton*.

Summary

1. The plants which were used as materials in the present investigation are 26 species and 6 formae of *Potamogeton*.

2. The chromosomes numbers determined by the author are as follows: *Potamogeton orientalis* ($2n=26$), *P. sibiricus* ($2n=28$), *P. zosterifolium* ($2n=28$), *P. porsildiorum* ($2n=28$), *P. cristatus* ($2n=28$), *P. miduhikimo* ($2n=28$), *P. limosellifolius* ($2n=28$), *P. asiaticus* ($2n=28$), *P. Fauriei* ($2n=27$), *P. Kamogawaensis* ($2n=27$), *P. numasakianus* ($2n=28$), *P. Friyeri* ($2n=42$), *P. subsesilifolius* ($2n=42$), *P. torquatus* ($2n=42$), *P. miyakezimaensis* ($2n=52$), *P. subfluitans* ($2n=52$), *P. teganumensis* ($2n=52$), *P. leptocephalus* ($2n=52$), *P. anguillanus* ($2n=52$), *P. Tepperi* ($2n=52$), *P. Francheii* ($2n=52$), *P. longipetiolatus* ($2n=52$), *P. alatus* ($2n=52$), *P. polygonifolius* ($2n=52$), *P. malainoides* ($2n=52$), *P. grameans* ($2n=52$), *P. morongii* ($2n=52$), *P. Tenuifolius* ($2n=52$), *P. filiformis* ($2n=78$), *P. vaginatus* ($2n=78$), *P. flabellatus* ($2n=78$).

In the following 17 species the reports of other investigations are confirmed by the author: *P. oxyphyllus* ($2n=26$), *P. panormitanus* ($2n=26$), *P. pusillus* ($2n=26$), *P. monoginus* ($2n=28$), *P. vaseyi* ($2n=28$), *P. apertus* ($2n=42$), *P. malianus* ($2n=52$), *P. dentatus* ($2n=52$), *P. nipponicus* ($2n=52$), *P. perfoliatus* ($2n=52$), *P. crispus* ($2n=52$), *P. praelongus* ($2n=52$), *P. Maackianus* ($2n=52$), *P. biwaensis* ($2n=52$), *P. distinctus* ($2n=52$), *P. natans* ($2n=52$), and *P. pectinatus* ($2n=78$).

The karyotypes and meiosis in all these species were observed. Moreover, some interspecific

hybrids were cytologically studied.

3. From the view point of phylogeny and cytology, the species of this genus studied can be classified into two groups : (a) the 7 chromosomic group, (b) the 13 chromosomic group.

The following species being to the 7 chromosomic group: *P. monoginus*, *P. crispus*, *P. vaseyi* and *P. numasakianus* are tetraploid ($2n=28$), while *P. apertus* and *P. Friyeri* are hexaploid ($2n=42$).

The following species belong to the 13 chromosomic group : *P. oxyphyllus*, *P. panormitanus*, *P. pusillus* and *P. orientalis* are $2n=26$. while *P. fluitanus*, *P. malaianus*, *P. dentatus*, *P. nipponicus*, *P. perfoliatus*, *P. crispus*, *P. praelongus*, *P. Maackianus*, *P. biwaensis*, *P. alpinus*, *P. anguillanus*, *P. distinctus* and *P. natans* have $2n=52$ chromosomes.

4. The following species are interspecific hybrids : *P. Fauriei*, *P. orientalis* *P. numasakianus*, *P. biwaensis*, *P. malainoides*, *P. anguillanus*, and *P. nipponicus*. In these interspecific hybrids, the irregularities in the meiosis, such as the occurrence of univalents, lagging of some univalents in the anaphase, and formation of restitution nuclei and dwarf nuclei, ets. are found to a greater or less extent.

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